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Research Article

Development and Validation of a UV-Vis Spectrophotometric Method for Determination of Pitavastatin in Bulk and Its Dosage Form

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ABSTRACT

The present work aimed at developing Assay UV-Vis Spectrophotometric method for determination of Pitavastatin in Pitavastatin Tablets. It exhibited absorption maxima at 245 nm in methanol water (50:50). The method is specific there is no interference with the blank. The linearity within the range of 2.042 mcg/ml to 12.252mcg/ml with regression coefficient of 0.999. The % recovery within the range of 99.21 to 101.11 and the standard deviation and %relative standard deviations are less than 2. The method was found to be precise according to the method precision data, intermediate precision data and intraday precision data with the standard deviation and % relative standard deviation less than 2. The method was rugged and robust and % relative standard deviation less than 2. The proposed method was found suitable, rapid, cost effective, accurate, precise, robust and rugged for Assay routine analysis of Pitavastatin in its marketed dosage form.

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INTRODUCTION [1,4]

Pitavastatin is a drug of choice in lipid-lowering agent that works to control the synthesis of cholesterol via competitive inhibition of the liver enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Chemically it is a (+) monocalcium bis {(3R, 5S, 6E)-7- [2-cyclopropyl-4-(4- fluorophenyl)-3-quinolyl]-3, 5dihydroxy-6-heptenoate}, physically available its white to pale-yellow powder sparingly soluble in water and freely soluble in DMF, Methanol and Ethanol with Pka-4.13 and structure of Pitavastatin as follows.

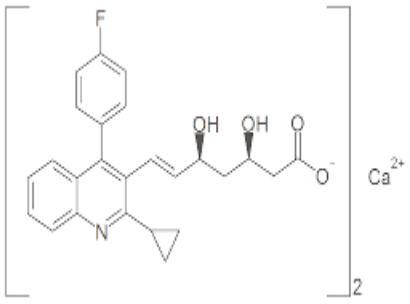

Fig No. 1- **Structure of Pitavastatin Calcium**

In literature review a very few works was carried by UV spectroscopy **S**o attempt was made to made develop UV-Vis Spectroscopic method for determination of Pitavastatin in Pitavastatin marketed formulations.

EXPERIMENTAL WORK

Optimization

Solubility: Solubility of the drug was studied using various solvents such as distilled water, methanol, 0.1N HCl, 0.1N NaOH and DMSO etc.

Determination of Working Wavelength: In order to ascertain the wavelength of maximum absorption (λ_{max}) of each drug, different solutions of the drug (2.042 mcg/ml to 12.252mcg/ml) in methanol: water (50:50) were scanned using spectrophotometer within the wavelength region of 200 – 400 nm against methanol: water (50:50) as blank.

Method:

Preparation of Stock Solutions: Standard stock solutions were prepared by dissolving 10 mg of each drug separately in 10 ml of methanol: water (50:50) to get concentration of 1mg/ml (1000 µg/ml) solutions.

Calibration Curve: The prepared stock solutions were further diluted with methanol: water (90:10) to get working standard solutions of 100 µg/ml of the selected drugs. To construct Beer's law plot for pure drug, different aliquots of drug was taken and diluted to 10 ml with methanol: water (50:50). The absorbance of each solution was measured at their respective λ_{max} against methanol: water (50:50) as blank. The calibration curves were plotted by taking concentration of drug on x-axis and absorbance on y-axis.

Estimation of Drug in their Dosage Forms: Twenty tablets were weighed, average weight determined and crushed to fine powders. An accurately weighed sample equivalent to 8 mg of the drug was transferred to a 100 ml volumetric flask. The drug was extracted with diluent and sonicate for 15 minutes with intermediate shaking and then volume was made up to the mark, using the same solvent. After appropriate dilution (within their linearity range), absorbance of each sample solution was recorded at respective λ_{max} and concentration of drugs in the samples were calculated.

Validation [2,3]

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples within the linearity range and added to the pre-analysed formulation of concentration 8 µg/ml and from that percentage recovery values were calculated.

Precision: The precision of the proposed method was ascertained by actual determination of six replicates of fixed concentration of the drugs within the Beer's range and finding out the absorbance by the proposed method. From the absorbance, Mean, Standard deviation and %RSD were calculated. Different parameters included for precision study were repeatability, intraday and interday precision

Ruggedness: Ruggedness is the degree of reproducibility of the results obtained under a verity of conditions. These conditions included different analysts and different instruments. The data was subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD.

Robustness: Robustness of the method was studied by deliberate variations of the analytical parameter such as solvent composition. The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD.

Detection Limit and Quantification Limit:

Calibration curve was plotted by using concentration in the expected detection limit range (0.1-5 µg/ml) for the drug. The standard deviation of y-intercept of regression line were determined and substituted in the following equation for the determination of detection limit and quantification limits.

Detection limit = 3.3 σ/s and

Quantification limit = 10 σ/s

Where σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve.

RESULTS AND DISCUSSION

Optimization.

Solubility of Pitavastatin

Pitavastatin was sparingly soluble in water but soluble in methanol, ethanol, 0.1N NaOH, 0.1 HCl, and insoluble in 0.1N NaOH etc.

Method

Determination of Working Wavelength: In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, different concentrations of Pitavastatin (2.042 mcg/ml to 12.252mcg/ml) in methanol and water (50:50) were scanned using UV-VIS spectrophotometer within the wavelength region of 200 – 400 nm against methanol and water (50:50) as blank. The resulting spectra were shown in Fig. 2 and the absorption curve showed characteristic absorption maxima at 245 nm for Pitavastatin.

CALIBRATION CURVE FOR PITAVASATIN

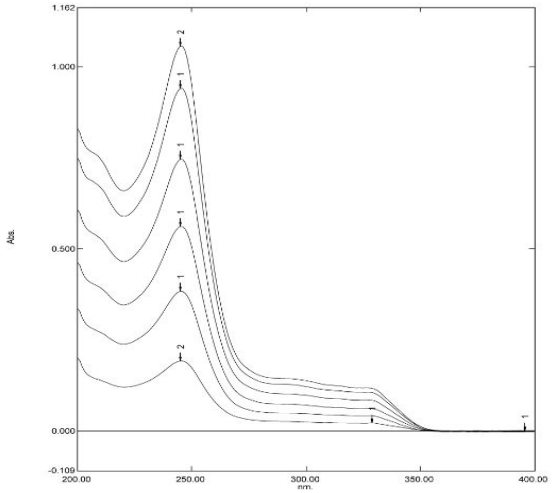

Fig.2: **UV-Vis Spectra of Pitavastatin**

Preparation of Calibration Curve: For preparation of calibration curve of Pitavastatin a stock solution of 1000µg/ml was prepared. From it different concentrations ranging from 2.042 mcg/ml to 12.252mcg/ml were prepared. Then the samples were scanned using UV-VIS Spectrophotometer and their absorbances were noted, which are given in the table below.

Table 1: Calibration Table of the UV-Vis Spectrophotometric Method for Pitavastatin

Conc. (mcg/ml)	Abs1	Abs2	Abs3	Abs4	Abs5	Abs6	mean	stdev	%RSD
2.042	0.192	0.194	0.192	0.196	0.192	0.192	0.192	0.193	0.002
4.084	0.383	0.381	0.385	0.387	0.383	0.385	0.386	0.384	0.002
6.126	0.561	0.56	0.561	0.563	0.565	0.563	0.566	0.563	0.002
8.168	0.748	0.745	0.741	0.748	0.745	0.746	0.742	0.745	0.003
10.21	0.94	0.91	0.948	0.93	0.95	0.96	0.94	0.940	0.016
12.252	1.15	1.159	1.112	1.15	1.17	1.18	1.15	1.153	0.021

A calibration curve was plotted using these readings taking concentration on X-axis and absorbance on Y-axis.

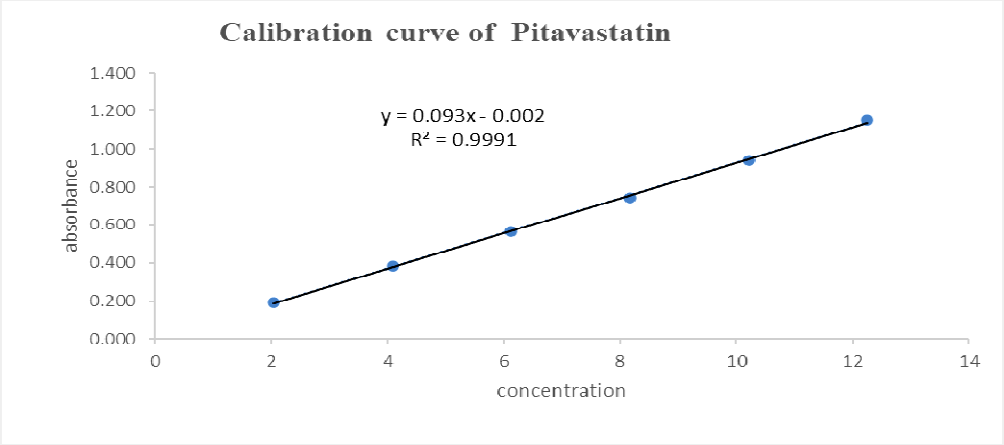

Fig no-2. Calibration curve for Pitavastatin

From the calibration curve it was found that it shows linearity in the range of 2.042 mcg/ml to 12.252mcg/ml with regression coefficient 0.999

Table 2: Optical Characteristics of Pitavastatin

Beer's Law limit (µg/ml)	2.042-12.252µg/ml
Sandell's sensitivity (µg/cm^3 / 0.001absorbance unit)	0.0202
Molar extinction coefficient	157429
% Relative standard deviation	0.021
Correlation coefficient	0.999
Regression equation (Y)	Y=0.093x-0.002
Slope (a)	0.093
Intercept (b)	-0.002

Validation

Table 3: Accuracy Data of the UV-Vis Spectrophotometric Method for Pitavastatin

Level	Amount	Abs	Amount	%	Mean	SD	% RSD
Level 1 (80%)	1.61	0.612	1.64	101.74	100.79	0.88	0.87
	1.60	0.601	1.61	100.63			
	1.60	0.599	1.60	100.00			
Level 2 (100%)	2.100	0.780	2.09	99.52	99.21	0.27	0.27
	2.120	0.785	2.10	99.06			
	2.130	0.789	2.11	99.06			
Level 3 (120%)	2.40	0.902	2.42	100.83	101.11	0.49	0.48
	2.40	0.911	2.44	101.67			
	2.40	0.904	2.42	100.83			
Overall Mean						**100.37**	
Overall SD						**1.02**	
Overall % RSD						**1.02**	

Table 4: Precision Data Showing Repeatability of the UV-Vis Spectrophotometric Method for Pitavastatin

Sr. No	Absorbance	% Assay of Pitavastatin
1	0.756	100.22
2	0.752	99.39
3	0.756	99.40
4	0.758	100.18
5	0.759	100.80
6	0.756	100.22
Mean		**100.13**
SD		**0.629**
%RSD		**0.63**

Table 5: Interday Precision Data of the UV-Vis Spectrophotometric Method for Pitavasatin

% Assay of Pitavastatin			
Sr. No.	**Absorbance**	**Intermediate precision**	**Method Precision**
1	0.758	101.59	100.22
2	0.756	101.01	99.39
3	0.752	100.18	99.40
4	0.756	100.19	100.18
5	0.758	100.98	100.80
6	0.759	101.60	100.22
Mean		**100.93**	**100.13**
SD		**0.633**	**0.629**
%RSD		**0.63**	**0.63**
Overall Mean		100.53	
Over all SD		0.73	
Overall %RSD		0.73	
Analyst		Analyst II	Analyst I
UV-Vi spectrophotometer ID		UV-1700	UV-1800
Date		01.06.2022	03.06.2022

Table 6: Robustness Data of the of Pitavastatin

Parameter	%Assay	% RSD	Overall% RSD Of Assay
Plus, Wavelength (247nm)	100.73	1.00	100.48
Minus Wavelength (243 nm)	100.21	0.14	100.30
Plus, Organic Methanol: Water (55: 45)	100.67	0.48	100.46
Minus Organic Methanol: Water (45: 55)	99.81	0.50	100.17

Limit of Detection (LOD):

The LOD for Pitavastatin was found to be 0.5µg/ml.

Limit of Quantitation (LOQ):

The LOQ for Pitavastatin was found to be 1.65µg/ml.

CONCLUSION

The ICH guidelines work with an objective to increase international harmonization of technical requirements to ensure that safe, effective, and high-quality medicines are developed and registered in the most efficient and cost-effective manner. The present work aimed at developing Assay UV-Vis Spectrophotometric method for Pitavastatin in pitavastatin Tablets. It exhibited absorption maxima at 245 nm in methanol water (50:50). The method is specific there is no interference with the blank. The linearity within the range of 2.042 µg/ml to 12.252 µg/ml with regression coefficient of 0.999. The %recovery within the range of 99.21 to 101.11 and the standard deviation and %relative standard deviations are less than 2. The method was found to be precise according to the repeatability data, intraday precision data and interday precision data with the standard deviation and % relative standard deviation less than 2. The method was rugged, robust and the standard deviation and % relative standard deviation less than 2. The proposed method was found suitable, rapid, cost effective, accurate, precise, robust and rugged for Assay routine analysis of Pitavastatin in its marketed dosage form.

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